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Effect of an Herbal Supplement (Citrus Bergamia, Cinnamomum Cassia, Morus Alba L., Lagerstroemia Speciosa Pars., and Sesamum Indicum) on Diet-Induced Dyslipidemia in Hamsters

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Abstract

Background: *Cholesterol levels in the blood are highly correlated with cardiovascular disease. Herbal extracts such as bergamot, cinnamon, mulberry leaves, and banaba leaves are rich in bioactive compounds, including polyphenols, flavonoids, or phytic acid, which demonstrate potential lipid-regulating properties. Thus, this study investigates the effects of a herbal extract supplement on lipid modulation.*

Methods: *In this study, Syrian hamsters were induced with hyperlipidemia using a high-cholesterol diet (HCD) and simultaneously supplemented with herbal supplements (HS) at doses of 0, 250.64, 504.20, and 1253.04 mg/kg, with nine hamsters in each group. After six weeks of intervention, lipid and cholesterol accumulation in the blood, liver, and feces was measured.*

Results: *The results indicated that, following the six-week supplementation of HS, hyperlipidemic hamsters showed significant reductions in blood triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and the LDL-C/ high-density lipoprotein cholesterol (HDL-C) ratio, while the HDL-C/TC ratio significantly increased compared to the HCD control group. Additionally, TG and TC levels in both the liver and feces were significantly reduced.*

Conclusion: *Overall, HS demonstrated lipid-regulating effects by decreasing cholesterol accumulation in the blood, liver, and feces, making it suitable for development as a dietary supplement for lipid modulation.*

Keywords: *Hyperlipidemia, Citrus Bergamia, Cinnamomum Cassia, Morus Alba L., Lagerstroemia Speciosa Pars.*

INTRODUCTION

Cardiovascular disease (CVD) is one of the prevalent diseases in humans. According to the statistics from The Global Cardiovascular Risk Consortium, the incidence rates of cardiovascular disease in males and females are 52.6% and 57.2% respectively, while the 10-year mortality rates after suffering from CVD reach 22.2% and 19.1% (Global Cardiovascular Risk et al., 2023). The factors contributing to CVD encompass dietary imbalances, tobacco use, alcohol consumption, stress, obesity, lack of physical activity, hypertension, elevated blood sugar levels, hyperlipidemia, and genetic predisposition (Sharifi-Rad et al., 2020). The concentrations of cholesterol in the blood, such as low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), are correlated with CVD (Soppert, Lehrke, Marx, Jankowski, & Noels, 2020). When the accumulation of triglycerides (TG) in the blood exceeds 150 mg/dL, it also exacerbates the risk of CVD (Aberra et al., 2020).

Bergamot (*Citrus bergamia*) is a citrus fruit native to the Mediterranean region, particularly Calabria in the southern part of Italy. It has garnered growing interest owing to its abundant composition of bioactive molecules and distinctive profile characterized by flavonoid glycosides, including neoeriocitrin, neohesperidin, naringin (Pierdomenico et al., 2023). Previous research showed that in individuals with LDL-C levels ranging from 116-190 mg/dL, continuous supplementation with a compound containing 200 mg of bergamot extract for 8 and 16 weeks resulted in a significant reduction in LDL-C by 18.2% and 23.4% respectively, along

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with a significant increase in HDL-C by 21.3% and 24.8 (Folco, Vallecorsa, Massari, & Tubili, 2023). This demonstrates the potential of bergamot extract in regulating blood cholesterol concentrations.

The organic material cinnamon (*Cinnamomum cassia*), extracted from the inner bark of trees, is a widely used spice known for its numerous benefits. Represented by around 250 species found across Asia, Australia, and South America. Cinnamon extract offers a more concentrated flavor source than ground cinnamon (Alsoodeeri, Alqabbani, & Aldossari, 2020). It primarily consists of vital oils and derivatives like cinnamaldehyde, cinnamic acid, and cinnamate, which contribute to its natural antioxidant, anti-inflammatory, antidiabetic, and cholesterol-lowering properties (Błaszczyk, Rosiak, & Kałużna-Czaplińska, 2021). A studies has shown that in streptozotocin-induced rats with energy metabolism disturbances, continuous supplementation of cinnamon extract (300, 400, 500 mg/kg) for 8 weeks significantly reduces blood total cholesterol (TC), TG, and LDL-C concentrations (Vijayakumar et al., 2023). This indicates that cinnamon extract has beneficial effects on lipid metabolism.

The leaves of the mulberry tree (*Morus alba L.*) are extensively employed both as a traditional Chinese medicine and as a functional food for diabetes management (Zhao et al., 2022). 1-Deoxynojirimycin (1-DNJ), a well-established alpha-glucosidase inhibitor, is abundant in mulberry leaves. Research has indicated that treatment with purified 1-DNJ may enhance insulin sensitivity in streptozotocin-induced diabetic rats and *db/db* mice (Kang, Park, & Lee, 2022). Previous studies also demonstrated that mulberry leaf extract can improve hepatic cholesterol accumulation in oleate-induced nonalcoholic steatohepatitis rats by downregulating the expression of sterol regulatory element binding protein 2 (SREBP2) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, suggesting that mulberry leaf extract has the potential to modulate cholesterol biosynthesis (Hu et al., 2020).

The leaves of *Lagerstroemia speciosa (L.)* are indigenous to Southeast Asia and offer both medicinal and horticultural benefits. Their bioactive phytochemicals exhibit a variety of properties, including hypoglycemic, antibacterial, anti-inflammatory, antioxidant, and hepatoprotective effects (Ganesan & Sujatha, 2024). Corosolic acid and tannins are recognized as bioactive compounds in *L. speciosa* that aid in lowering glucose levels (Yusakul, Saensom, Mitsantia, Pengdee, & Putalun, 2020). Extracts from *L. speciosa* have been shown to improve metabolic parameters, including fasting blood glucose, HbA1c, body weight, and cholesterol levels, contributing to the management of metabolic syndrome (Hassan, 2023).

Despite the development of various medications for metabolic disorders, patients often encounter additional challenges due to side effects, such as gastrointestinal issues, heart failure, weight gain, edema, impaired kidney function, pancreatitis, and genital infections (Lee, Noh, Lim, & Kim, 2021). In contrast, natural plant extracts generally exhibit fewer side effects, and studies indicate that these extracts can assist in the regulation of lipid metabolism. Therefore, this study used a high-cholesterol diet-induced hyperlipidemia model to investigate the effects of herbal compound supplements on lipid and cholesterol accumulation in the blood and liver.

MATERIALS AND METHODS

Experimental Supplement

The herbal supplement (HS) was provided by HealthTake Co., Ltd. (508 mg per tablet). The formula contained herbal extract (*Citrus bergamia*, *Cinnamomum cassia*, *Morus alba L.*, *Lagerstroemia speciosa Pars.*, and *Sesamum indicum*), vitamin C, yeast chromium, zinc glycinate, vitamin E.

Animal and Study Design

Male Syrian hamsters were purchased from the National Laboratory Animal Center (Taipei, Taiwan). A total of 45 hamsters, aged 8 weeks, were housed at the Animal Facility of National Taiwan Sport University. The study was approved by the IACUC of the National Taiwan Sport University (No. 11105). The animal facility maintained a temperature of 22 ± 2°C and a 12-hour light-dark cycle (lights on at 6 AM and off at 6 PM). All hamsters were allowed to access the experimental diet and sterilized water freely throughout the experiment. After one week of acclimatization, the hamsters were divided into five groups, each consisting of 9 hamsters: (1) ND group, (2) HCD control group, (3) HCD + HS-1X group, (4) HCD + HS-2X group, and (5) HCD + HS-5X group. The ND group was fed a standard AIN-93 diet, and four HCD groups were fed a 0.2% cholesterol diet based on the AIN-93 diet. The compositions of the two diets are shown in Table 1. The HS treatment groups were orally gavage daily with HS at doses of 1X, 2X, and 5X (250.64, 504.20, or 1253.04 mg/kg, respectively) once per day. After 6 weeks of treatment, blood was collected from the hamsters after a 12-hour fast for biochemical analysis. Subsequently, the hamsters were euthanized using carbon dioxide, and their livers were collected, rinsed, and frozen at -80 ℃ for lipid analysis. Three days before euthanasia, feces were collected from the hamsters for feces lipid analysis.

Table 1. The composition of the diets used in the research

(g)	Normal diet	High cholesterol diet
Casein	141	140
Corn starch	620.7	558.7
Sucrose	100	100
Cellulose	50	50
Soybean oil	40	40
Lard	0	110
Mineral	35	35
Vitamin	10	10

L-cysteine	1.8	1.8
Choline bitartrate	2.5	2.5
Cholesterol	0	2.1
Total weight	1000	1065
Total calorie (kcal)	3930	4645

Blood Lipid Level Analysis

The blood samples were centrifuged at 3000×g for 15 minutes. The supernatant serum samples were collected, and serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed using an automated clinical chemistry analyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

Hepatic Lipid Level Analysis

Liver samples weighing 100 mg were homogenized in 1 mL of organic solvent (chloroform: isopropanol: NP40 = 7: 11: 0.1). The homogenates were then centrifuged at 15,000 × g for 10 minutes, and 400 µL of the supernatant was transferred to a vacuum machine and dried (50°C, 30 min). After drying, the samples were reconstituted with Cholesterol assay buffer and thoroughly mixed using ultrasonication and vortex. Subsequently, the determination of hepatic TG was conducted using the commercial GPO-PAP method kit (TR1697, RANDOX). The analysis of TC in the liver was performed using the commercial CHOD-PAP method kit (CH3810, RANDOX).

Fecal Lipid Level Analysis

After drying in an oven to constant weight, 100 mg of feces was extracted with 1 mL of extraction solvent (chloroform: methanol = 2: 1, v/v) and filtered through filter paper. The resulting filtrate was dried under vacuum and then reconstituted in DMSO, followed by thorough mixing using ultrasonication and vortex. Subsequently, the determination of fecal TG was conducted using the commercial GPO-PAP method kit (TR1697, RANDOX). The analysis of TC in feces was performed using the commercial CHOD-PAP method kit (CH3810, RANDOX).

Statistical Analysis

The results were presented as the mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test to compare groups. Statistical significance was considered at $p < 0.05$. All data analyses were performed using SAS statistics software (SAS Institute, Cary, NC, USA).

RESULTS

Effect of HS on the Body Weight in the Hamsters Receiving an HCD

The average weekly body weights of each group are shown in Table 2. There were no significant differences in body weight among the five groups at week 0 ($p > 0.05$). From week 2 to week 6, the body weights of the four groups fed with HCD were significantly higher than the ND group ($p < 0.05$). At week 6, the average body weights of the HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups were significantly increased by 1.27-fold, 1.23-fold, 1.24-fold, and 1.23-fold respectively compared to the ND group ($p < 0.0001$).

Table 2. Effect of the herbal supplement on the body weight in the hamsters receiving the HCD

Body weight (g)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
Week 0	105.13 ± 6.75^a	105.36 ± 7.95^a	105.84 ± 7.08^a	105.99 ± 8.92^a	105.64 ± 6.30^a
Week 1	107.21 ± 6.82^a	112.28 ± 8.22^a	111.96 ± 7.01^a	112.03 ± 8.94^a	111.51 ± 6.25^a
Week 2	109.28 ± 6.90^a	119.10 ± 8.24^b	119.38 ± 7.03^b	118.91 ± 8.89^b	119.83 ± 6.39^b
Week 3	111.47 ± 6.98^a	126.61 ± 8.27^b	125.78 ± 6.79^b	125.41 ± 9.53^b	125.77 ± 6.41^b
Week 4	113.70 ± 7.05^a	134.08 ± 8.34^b	132.67 ± 6.87^b	132.09 ± 9.82^b	132.47 ± 6.59^b
Week 5	115.86 ± 7.09^a	141.93 ± 8.53^b	139.28 ± 6.92^b	138.86 ± 9.83^b	137.34 ± 6.42^b
Week 6	118.10 ± 7.11^a	149.95 ± 8.76^b	146.39 ± 6.77^b	145.72 ± 9.79^b	145.07 ± 9.57^b

The reported values are the mean ± SD (n = 9). Different superscript letters (a and b) indicate significant differences between groups ($p < 0.05$).

Effect of HS on the Feed and Water Intake in the Hamsters Receiving an HCD

The feed intake of each group is shown in Table 3. At week 6, the average daily intake was 9.56±0.61, 10.08±0.54, 10.00±0.59, 9.98±0.60, and 10.06±0.54 (g/day) for the ND, HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups, respectively. The average daily intake of the HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups was significantly increased by 1.05-fold, 1.05-fold, 1.04-fold, and 1.05-fold, respectively, compared to the ND group ($p < 0.0001$). The water intake of each group is shown in Table 4. During the six experimental weeks, the water intake of the five groups showed no significant difference ($p > 0.05$).

Table 3. Effect of the herbal supplement on the feed intake in the hamsters receiving the HCD

Feed intake (g/hamster/day)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
Week 1	7.90 ± 0.64[a]	8.61 ± 0.82[b]	8.58 ± 1.03[b]	8.61 ± 0.90[b]	8.50 ± 0.90[b]
Week 2	7.86 ± 0.65[a]	8.70 ± 0.91[b]	8.68 ± 0.73[b]	8.87 ± 1.12[b]	8.73 ± 1.08[b]
Week 3	8.16 ± 1.58[a]	8.69 ± 1.72[b]	8.84 ± 1.71[b]	8.73 ± 1.65[b]	8.70 ± 1.94[b]
Week 4	9.15 ± 0.70[a]	9.83 ± 0.59[b]	9.91 ± 0.62[b]	9.84 ± 0.91[b]	9.90 ± 0.57[b]
Week 5	9.16 ± 1.80[a]	9.31 ± 1.93[b]	9.43 ± 1.87[b]	9.34 ± 2.02[b]	9.40 ± 2.01[b]
Week 6	9.56 ± 0.61[a]	10.08 ± 0.54[b]	10.00 ± 0.59[b]	9.98 ± 0.60[b]	10.06 ± 0.54[b]

The reported values are the mean ± SD (n = 9). Different superscript letters (a and b) indicate significant differences between groups ($p < 0.05$).

Table 4. Effect of the herbal supplement on the water intake in the hamsters receiving the HCD.

Water intake (g/hamster/day)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
Week 1	13.59 ± 1.63[a]	13.63 ± 1.91[a]	13.68 ± 1.75[a]	13.70 ± 1.87[a]	13.49 ± 1.82[a]
Week 2	13.29 ± 1.49[a]	13.56 ± 1.74[a]	14.54 ± 1.73[a]	14.47 ± 1.53[a]	13.84 ± 1.07[a]
Week 3	14.66 ± 1.82[a]	14.54 ± 1.73[a]	14.82 ± 1.68[a]	14.98 ± 1.65[a]	14.71 ± 1.62[a]
Week 4	14.45 ± 1.60[a]	14.47 ± 1.53[a]	14.40 ± 1.55[a]	14.42 ± 1.27[a]	14.46 ± 1.72[a]
Week 5	13.99 ± 1.48[a]	13.84 ± 1.07[a]	13.96 ± 1.55[a]	13.91 ± 1.61[a]	13.82 ± 1.20[a]
Week 6	14.38 ± 1.88[a]	14.44 ± 1.75[a]	14.05 ± 1.63[a]	13.95 ± 1.76[a]	13.91 ± 1.76[a]

The reported values are the mean ± SD (n = 9). There is no significant difference of the above data between the groups.

Effect of HS on the Blood Lipid Levels in the Hamsters Receiving an HCD

The blood lipid levels of each group after 6 weeks of HS treatment are shown in Table 5. The serum TG levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 2.71-fold, 2.16-fold, 1.93-fold, and 1.81-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum TG concentrations in the 1X, 2X, and 5X groups decreased significantly by 20.30%, 28.95%, and 33.46% respectively compared to the HCD group control ($p < 0.0001$).

Table 5. Effect of the herbal supplement on the blood lipids in the hamsters receiving the HCD

Blood lipids (mg/dL)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
TG	98 ± 7[a]	266 ± 17[e]	212 ± 7[d]	189 ± 9[c]	177 ± 8[b]
TC	121 ± 8[a]	323 ± 11[d]	291 ± 14[c]	279 ± 14[c]	265 ± 15[b]
HDL-C	60 ± 3[a]	93 ± 6[b]	91 ± 5[b]	92 ± 6[b]	90 ± 6[b]
LDL-C	38 ± 3[a]	152 ± 9[d]	130 ± 10[c]	126 ± 6[c]	117 ± 8[b]
LDL-C/HDL-C ratio	0.64 ± 0.02[a]	1.64 ± 0.08[e]	1.44 ± 0.04[d]	1.37 ± 0.04[c]	1.29 ± 0.04[b]
HDL-C/TC ratio	0.49 ± 0.01[a]	0.29 ± 0.01[e]	0.31 ± 0.01[d]	0.33 ± 0.01[c]	0.34 ± 0.01[b]

The reported values are the mean ± SD (n = 9). Different superscript letters (a, b, c, d, and e) indicate significant differences between groups ($p < 0.05$). TG, triglyceride. TC, total cholesterol. HDL-C, high density lipoprotein-cholesterol. LDL-C, low-density lipoprotein-cholesterol.

The serum TC levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 2.67-fold, 2.40-fold, 2.31-fold, and 2.19-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum TC concentrations in the 1X, 2X, and 5X groups decreased significantly by 9.91%, 13.62%, and 17.86% respectively compared to the HCD group control ($p < 0.0001$).

The serum HDL-C levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 1.55-fold, 1.52-fold, 1.53-fold, and 1.50-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum HDL-C concentrations in the 1X, 2X, and 5X groups showed no significant difference compared to the HCD group control ($p > 0.05$).

The serum LDL-C levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 4.00-fold, 3.42-fold, 3.32-fold, and 3.08-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum LDL-C concentrations in the 1X, 2X, and 5X groups decreased significantly by 14.47%, 17.11%, and 23.03% respectively compared to the HCD group control ($p < 0.0001$).

The serum LDL-C/HDL-C ratios of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 2.56-fold, 2.25-fold, 2.14-fold, and 2.02-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum LDL-C/HDL-C ratios in the 1X, 2X, and 5X groups decreased significantly by 12.20%, 16.46%, and 21.34% respectively compared to the HCD group control ($p < 0.0001$).

The serum LDL-C/TC ratios of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant decreases compared to the ND group by 40.82%, 36.73%, 32.65%, and 30.61% respectively ($p < 0.0001$). After 6 weeks of HS treatment, the serum LDL-C/TC ratios in the 1X, 2X, and 5X groups decreased significantly by 1.07-fold, 1.14-fold, and 1.17-fold respectively compared to the HCD group control ($p < 0.0001$).

Effect of HS on the Hepatic Lipids Level in the Hamsters Receiving an HCD

The hepatic lipid levels of each group after 6 weeks of HS treatment are shown in Table 6. The hepatic TG levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 3.49-fold, 3.21-fold, 2.97-fold, and 2.72-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the hepatic TG concentrations in the 1X, 2X, and 5X groups decreased significantly by 7.98%, 14.88%, and 21.93% respectively compared to the HCD group control ($p < 0.0001$).

The hepatic TC levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 3.46-fold, 3.14-fold, 2.74-fold, and 2.46-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the hepatic TC concentrations in the 1X, 2X, and 5X groups decreased significantly by 9.41%, 20.95%, and 29.05% respectively compared to the HCD group control ($p < 0.0001$).

Table 6. Effect of the herbal supplement on the hepatic lipids in the hamsters receiving the HCD

Hepatic lipids (mg/g wet liver)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
TG	5.53 ± 1.43^{a}	19.29 ± 1.29^{e}	17.75 ± 1.29^{d}	16.42 ± 0.54^{c}	15.06 ± 0.77^{b}
TC	3.53 ± 0.61^{a}	12.22 ± 0.62^{e}	11.07 ± 0.67^{d}	9.66 ± 0.72^{c}	8.67 ± 0.76^{b}

The reported values are the mean ± SD (n = 9). Different superscript letters (a, b, c, d, and e) indicate significant differences between groups ($p < 0.05$). TG, triglyceride. TC, total cholesterol.

Effect of HS on the Fecal Lipids Level in the Hamsters Receiving an HCD

The fecal lipid levels of each group after 6 weeks of HS treatment are shown in Table 7. The fecal TG levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 1.57-fold, 2.49-fold, 2.81-fold, and 3.07-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the fecal TG concentrations in the 1X, 2X, and 5X groups increased significantly by 1.59-fold, 1.79-fold, and 1.95-fold respectively compared to the HCD group control ($p < 0.0001$).

The hepatic TC levels of HCD, HCD + HS-1X, HCD + HS-2X, and HCD + HS-5X groups exhibited significant increases compared to the ND group by 4.17-fold, 5.07-fold, 5.90-fold, and 6.72-fold respectively ($p < 0.0001$). After 6 weeks of HS treatment, the hepatic TC concentrations in the 1X, 2X, and 5X groups increased significantly by 1.22-fold ($p = 0.002$), 1.42-fold, and 1.61-fold respectively compared to the HCD group control ($p < 0.0001$).

Table 7. Effect of the herbal supplement on the fecal lipids in the hamsters receiving the HCD.

Fecal lipids (mg/g wet feces)	ND	HCD			
		Control	HS-1X	HS-2X	HS-5X
TG	5.53 ± 1.43^{a}	19.29 ± 1.29^{e}	17.75 ± 1.29^{d}	16.42 ± 0.54^{c}	15.06 ± 0.77^{b}
TC	3.53 ± 0.61^{a}	12.22 ± 0.62^{e}	11.07 ± 0.67^{d}	9.66 ± 0.72^{c}	8.67 ± 0.76^{b}

The reported values are the mean ± SD (n = 9). Different superscript letters (a, b, c, d, and e) indicate significant differences between groups ($p < 0.05$). TG, triglyceride. TC, total cholesterol.

DISCUSSION

Long-term hyperlipidemia increased the risk of cardiovascular diseases, while plant extracts possessed the potential to modulate lipid levels. This study employed a diet-induced hyperlipidemic hamster model to investigate the effects of HS on lipid metabolism *in vivo*. The results demonstrated that HS could reduce the accumulation of TG, TC, and LDL-C in the blood and decreased TG and TC levels in the liver and feces, indicating that HS had lipid-regulating properties.

Dyslipidemia is characterized by an abnormal concentration of lipids in the bloodstream, including TGs, cholesterol, and phospholipids. Specifically, it involves elevated levels of TC, LDL-C, and TG, along with decreased levels of HDL-C (Sharifi-Rad et al., 2020). The results of this study indicate that after six weeks of supplementation with HS, hamsters induced with HCD exhibited significant reductions in blood TG, TC, LDL-C, and the LDL-C/HDL-C ratio, while the HDL-C/TC ratio significantly increased ($p < 0.05$) (Table 5). Previous literature shows that individuals with hyperlipidemia (5.4-7.0 mmol/L) who supplemented with bergamot extract (500 mg/day) for four weeks experienced significant decreases in blood TC, LDL-C, LDL-C/HDL-C ratio, and TC/HDL-C levels (Rondanelli et al., 2021). Another study demonstrated that diabetic patients who were continuously supplemented with 1000 mg of mulberry and banaba leaf extracts daily for four months achieved significant reductions in blood TC (Hassan, 2023). Additionally, research indicated that rats with HCD-induced hyperlipidemia supplemented with cinnamon powder (2 and 4 g/kg) for 30 consecutive days showed significant decreases in serum TC, TG, and LDL-C levels (Alsoodeeri et al., 2020). These findings were consistent with our study, suggesting that the lipid-regulating effects of HS were related to the functional plant extracts it contains.

In the liver, TGs are sourced from dietary intake, *de novo* fatty acid synthesis, and free fatty acids released from adipose tissue. The primary transcription factor that activates the genes essential for *de novo* fatty acid synthesis and the initial step of triglyceride synthesis is SREBP-1c (Berger & Moon, 2021). In this study, after four weeks of supplementation with HS, hyperlipidemic hamsters exhibited significant reductions in TG and TC in both liver and fecal samples ($p < 0.05$) (Tables 6 and 7). Bergamot extract is rich in flavonoids such as neoeriocitrin, naringin, neohesperidin, melitidin, and brutieridin. Notably, neohesperidin has been shown to lower TC levels in HepG2 cells by inhibiting the expression of HMG-CoA reductase and increasing AMP-activated protein kinase (AMPK) phosphorylation, thereby reducing cholesterol synthesis (Huang, Tocmo, Nauman, Haughan, & Johnson, 2021). The bioactive compounds in cinnamon have demonstrated the ability to decrease cholesterol and fatty acid absorption in intestinal cells by inactivating the Niemann–Pick C1-like 1 and Cd36 mRNA receptors, respectively. Furthermore, cinnamon could downregulate chylomicron synthesis by lowering levels of microsomal triglyceride transfer protein (MTTP) and inhibiting Apo B48 secretion from enterocytes (Silva, Bernardo, Singh, & de Mesquita, 2022). Additionally, mulberry leaf extract has been shown to inhibit the expression of the *SREBP-1c* gene in HepG2 cells, which suppresses hepatic lipogenesis, while also increasing *CYP7A1* gene expression to promote the conversion of cholesterol to bile acids (Li et al., 2023). Corosolic acid, abundant in banaba leaf, has also been demonstrated to reduce TC and TG accumulation in HepG2 cells, inhibiting the expression of *SREBP-1C, fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1),* and *HMG-CoA reductase*, while activating *AMPK* and *ACC* gene expression, indicating that banaba leaf extract could inhibit hepatic cholesterol production (Zhang et al., 2020). These findings suggested that HS could reduce cholesterol accumulation in the liver and feces, likely by modulating the expression of genes involved in cholesterol biosynthesis to suppress hepatic cholesterol production.

High cholesterol in the diet was likely to increase cardiovascular disease risk factors. All dietary patterns recommended by the U.S. Department of Agriculture in the 2015 to 2020 report contained less than 300 mg/d of cholesterol across calorie levels of up to 3200 kcal/day (Carson et al., 2020). Reducing dietary cholesterol intake could decrease the accumulation of cholesterol in the body. Furthermore, medications that inhibited cholesterol synthesis could achieve effective lipid regulation. Statins, a commonly used clinical medication, acted as inhibitors of HMG-CoA) reductase, an enzyme crucial for cholesterol synthesis. However, these medications are associated with side effects related to muscle and liver toxicity (Yu et al., 2020). Plant extracts have been shown to regulate lipid levels and typically exhibit fewer side effects compared to pharmaceutical drugs (Lee et al., 2021). Thus, they are suitable for development as functional ingredients. In this study, hamsters fed an HCD exhibited significantly higher food intake and body weight compared to those on an ND (Tables 2 and 3), indicating the impact of a high-calorie, high-cholesterol diet on body weight. The group receiving the HS showed no significant differences in feed intake or body weight compared to the HCD control group ($p > 0.05$). Additionally, there were no significant differences in water intake among the five experimental groups ($p > 0.05$) (Table 4). These results suggest that HS did not adversely affect the growth parameters of hamsters. Previous literature indicated that supplementation with bergamot extract for eight weeks in patients with hyperlipidemia resulted in a 13.25% reduction in LDL-C, with no significant changes in body composition or adverse effects, demonstrating the lipid-lowering potential of bergamot extract without negative impacts on human health (Angelopoulos, 2023). An acute oral toxicity safety assessment revealed that mice supplemented with 2000 mg/kg of mulberry leaf extract showed no symptoms such as diarrhea, constipation, sleep disturbances, or vomiting at 1, 7, and 14 days post-supplementation, indicating the safety for the consumption of mulberry leaf extract (Sood, Shri, Singh,

F. Ahmad, & M. Attia, 2024). Another study found that rats continuously supplemented with 2000 mg/kg of mulberry leaf extract for four weeks exhibited no significant effects on body weight, liver indices (AST, ALT), renal indices (urea, creatinine), or electrolytes (sodium, potassium, chloride), suggesting the suitability of cinnamon extract for long-term supplementation (Ahmad et al., 2013). Furthermore, a study indicated that rats receiving 3000 mg/kg of banaba leaf extract for 14 consecutive days showed no significant effects on body weight, liver indices (AST, ALT), renal indices (urea, creatinine), or cholesterol parameters (TC, TG, LDL-C, HDL-C), confirming the safety of banaba leaf supplementation (Azad & Sunzida, 2015). Overall, bergamot extract, mulberry leaf extract, cinnamon extract, and banaba leaf extract were characterized by low toxicity and appropriate for long-term supplementation. The HS, which contained these four plant extracts, not only demonstrated lipid-regulating effects but also exhibited safety for supplementation, making it suitable for development as a functional dietary supplement.

CONCLUSION

Supplementation with HS for six weeks in hamster induced with HCD resulted in a reduction of cholesterol accumulation in the blood, liver, and feces, demonstrating a dose-dependent effect. HS exhibits lipid-regulating properties, making it suitable for development as a dietary supplement for lipid modulation. The effective dose was determined to be low, at 250.64 mg/kg for hamsters, which corresponds to an equivalent daily intake of 2032 mg of HS for a 60 kg human.

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